

Attorney Docket No.: 5207.520-US
Filed: December 2, 2003

US Application No.: 10/725,843
Examiner: Swope, Sheridan Lee

AMENDMENTS TO THE CLAIMS

What is Claimed is:

1. (Currently amended) A method for large-scale production of Factor VII or a Factor VII-related polypeptide, comprising the steps of:

- (i) propagating a large-scale culture of mammalian cells in medium lacking animal-derived components until the large-scale culture cells reach a second predetermined density, said large-scale culture having been created by a method comprising:
 - inoculating mammalian cells expressing Factor VII or a Factor VII-related polypeptide into a seed culture vessel containing medium lacking animal-derived components;
 - propagating the inoculated cells at least until the cells have reached a first predetermined density to form a seed culture,
 - transferring the seed culture to a large-scale culture vessel containing medium lacking animal derived components to form said large-scale culture;
- (ii) maintaining the large-scale culture in medium lacking animal-derived components under conditions appropriate for Factor VII expression, thereby causing the cells to produce Factor VII or a Factor VII-related polypeptide, and
- (iii) recovering the produced Factor VII or Factor VII-related polypeptide from the maintained culture.

2. (Currently Amended) A The method as defined in claim 1, wherein said mammalian cells are CHO cells.

3. (Currently Amended) A The method as defined in claim 1, wherein said Factor VII has a glycosylation pattern different from both Factor VII produced *in vivo* and Factor VII produced in BHK cells.

4. (Currently Amended) A The method as defined in claim 1, wherein said seed culture has been transferred to and propagated in one or more intermediate size vessels of progressively larger size

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prior to being transferred to said large scale vessel.

5. (Currently Amended) A The method as defined in claim 1, wherein the mammalian cells have been rendered suspension culture competent prior to being inoculated into the seed vessel.

6. (Currently Amended) A The method as defined in claim 1, wherein the mammalian cells have been adapted to grow in medium lacking animal-derived components prior to said inoculation.

7. Cancelled

8. (Currently Amended) A The method as defined in claim 7 1, wherein the large-scale culture is a macroporous carrier culture, said macroporous carrier bearing a positive charge.

9. (Currently Amended) A The method as defined in claim 1, wherein the maintaining step comprises regularly harvesting a portion of the supernatant of said large-scale culture and replacing it with fresh medium lacking animal-derived components.

10. (Currently Amended) A The method as defined in claim 4 9, wherein the maintaining step further comprises sedimentation of the cell-containing carriers prior to said harvesting.

11. (Currently Amended) A The method as defined in claim 1, wherein the maintaining step comprises cooling the culture to a pre-determined temperature before the sedimentation.

12. (Currently Amended) A The method as defined in claim 1, wherein the maintaining step comprises feeding said mammalian cells with glucose.

13. (Currently Amended) A The method as defined in claim 12, wherein said feeding comprises pulse feeding from 1 to 4 times per 24-hour period.

14. (Currently Amended) A The method as defined in claim 12, wherein said feeding comprises

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gradual or continuous introduction of glucose into the large scale culture.

~~15. (Currently amended) A method for large-scale production of Factor VII or a Factor VII-related polypeptide, comprising the steps of:~~

(i) maintaining a large-scale culture of mammalian cells having at a second predetermined density in medium lacking animal-derived components under conditions appropriate for Factor VII expression, thereby causing the cells to produce Factor VII or a Factor VII-related polypeptide, said large-scale culture having been created by a method comprising:

inoculating mammalian cells expressing Factor VII or a Factor VII-related polypeptide into a seed culture vessel containing medium lacking animal-derived components;

propagating the inoculated cells at least until the cells have reached a first predetermined density to form a seed culture,

transferring the seed culture to a large-scale culture vessel containing medium lacking animal derived components to form said large-scale culture; and

(ii) recovering produced Factor VII or Factor VII-related polypeptide from the maintained culture.

16. Cancelled

~~17. (Withdrawn) A Factor VII or Factor VII-related polypeptide produced by a method as defined in claim 1.~~

~~18. (Withdrawn) A Factor VII or Factor VII-related polypeptide produced by a method as defined in claim 15.~~

~~19. (Withdrawn) A Factor VII or Factor VII-related polypeptide produced by a method as defined in claim 16.~~

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20. (Withdrawn) A preparation comprising a plurality of Factor VII or Factor VII-related polypeptides expressed by recombinant BHK or CHO cells in the presence of media lacking animal-derived components (serum-free Factor VII), wherein the Factor VII or Factor VII-related polypeptides comprise N-linked oligosaccharides chains and the oligosaccharides exhibit a glycoform pattern differing from that of the same Factor VII or Factor VII-related polypeptide expressed by the same cells in the presence of serum (serum-raised Factor VII) and from that of Factor VII purified from human plasma (native Factor VII) and wherein a percentage of oligosaccharide chains in said preparation comprise at least one sialic acid moiety, said percentage being higher than that observed in serum-raised Factor VII preparations and lower than the corresponding percentage in native Factor VII preparations, said serum-free Factor VII preparation having a higher bioavailability than the bioavailability of a serum-raised Factor VII preparation.

21. (withdrawn) A preparation as defined in claim 20, wherein said serum-free Factor VII glycoform pattern exhibits an additional difference from that of Factor VII expressed by the same cells in the presence of serum (serum-raised Factor VII) and from that of Factor VII purified from human plasma (native Factor VII), said additional difference comprising one or more of the following:

(i) percentage of the oligosaccharide chains having a neutral charge, wherein the percentage of oligosaccharide chains of serum-free Factor VII having a neutral charge is lower than that of serum-raised Factor VII and higher than that of native Factor VII;

(ii) percentage of the oligosaccharide chains comprising at least one terminal galactose residue, wherein the percentage of oligosaccharide chains of serum-free Factor VII having at least one terminal galactose residue is lower than that of serum-raised Factor VII and higher than that of native Factor VII;

(iii) percentage of the oligosaccharide chains comprising at least one terminal N-acetylgalactosamine residue, wherein the percentage of oligosaccharide chains of serum-free Factor VII having at least one terminal N-acetyl galactose residue is lower than that of serum-raised Factor VII and higher than that of native Factor VII; and

(iv) percentage of the oligosaccharide chains comprising at least one uncapped antenna, wherein the percentage of oligosaccharide chains of serum-free Factor VII comprising at least one uncapped antenna is lower than that of serum-raised Factor VII and higher than that of native Factor VII.

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22. (Withdrawn) A pharmaceutical formulation comprising a polypeptide as defined in claim 17 and a pharmaceutically acceptable carrier or adjuvant.

23. (Withdrawn) A method for treating a Factor VII-responsive syndrome, the method comprising administering a pharmaceutical formulation as defined in claim 22 to a patient in need of such treatment, under conditions that result in a decrease in bleeding and/or an increase in blood clotting.

24. (Withdrawn) A method as defined in claim 23, wherein the syndrome is selected from the group consisting of haemophilia A, haemophilia B, Factor XI deficiency, Factor VII deficiency, thrombocytopenia, von Willebrand's disease, presence of a clotting factor inhibitor, surgery, trauma, and anticoagulant therapy.